

Syn-PER™ Synaptic Protein Extraction Reagent

87793

2382.0

Number	Description
87793	Syn-PER Synaptic Protein Extraction Reagent , 100mL, sufficient reagent to extract synaptic protein from ~10g of neuronal tissue and primary cultured neurons

Storage: Upon receipt store product at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Syn-PER Synaptic Protein Extraction Reagent extracts proteins expressed in the synapses of neuronal tissue and primary cultured neurons. Native pre- and post-synaptic proteins extracted by Syn-PER Reagent are suitable for downstream applications, including Western blots, enzymatic activity assays (e.g., phosphatase and kinase), protein-protein interaction studies and immunoprecipitations. Synaptosomes from prepared neuronal tissue can be used to study neurotransmitter release. Additionally, Syn-PER Reagent minimizes phosphoprotein degradation and does not require ultracentrifugation.

Important Product Information

- Syn-PER Reagent does not contain protease or phosphatase inhibitors. Add necessary inhibitors to Syn-PER Reagent immediately before use to a final inhibitor concentration of 1X (see Related Thermo Scientific Products).
- Syn-PER Reagent does not contain antimicrobial agents. Avoid contaminating the opened bottle.
- Perform all steps, including homogenization and centrifugation, at 4°C to reduce proteolysis, dephosphorylation and denaturation. Store samples and extracts on ice.

Additional Materials Required

- Ice-cold phosphate-buffered saline (PBS, Product No. 28372): 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2
- Refrigerated bench-top microcentrifuge
- Dounce tissue grinder
- EDTA-free protease, phosphatase or combination inhibitors (see the Thermo Scientific Related Products Section)

Procedure for Synaptic Protein Extraction from Neuronal Tissue

- Place the Dounce tissue grinder on ice before use.
 - Immediately before use, add inhibitors to the Syn-PER Reagent; add inhibitors only to the amount being used for the procedure and not to the stock solutions.
1. Weigh neuronal tissue samples. Add 10mL of Syn-PER Reagent per gram of tissue (e.g., 2mL of Syn-PER Reagent per 200mg of brain tissue).
 2. Perform Dounce homogenization on ice with ~10 slow strokes.
 3. Transfer homogenate to an appropriate centrifuge tube(s).
 4. Centrifuge the tube at 1200 × g for 10 minutes at 4°C. Discard the pellet and transfer supernatant to a new tube. If required, save a sample of the supernatant (homogenate) for analysis.
 5. Centrifuge supernatant at 15,000 × g for 20 minutes at 4°C.

- Remove the supernatant from the synaptosome pellet. If required, save the supernatant (cytosolic fraction) for analysis.
- Add 1-2mL of Syn-PER Reagent per gram of sample to suspend the synaptosome pellet (e.g., 500 μ L for 200-400mg of brain tissue).

Note: Recommended volumes should result in 3-4 μ g/ μ L of synaptic protein.

- Maintain the synaptosome suspension on ice until performing neurotransmitter release studies or downstream applications.

Note: The synaptosome suspension can be stored in 5% (v/v) DMSO at -80°C or in liquid nitrogen for extended periods of time; however, a substantial reduction in synaptosome viability occurs after prolonged storage. For best results, perform activity studies (e.g., calcium-dependent neurotransmitter release) with new synaptosomes.

Note: Further synaptosome suspension separation into pre- and post-synaptic protein fractions and synaptic vesicle proteins is done by applying the synaptosome suspension onto a discontinuous sucrose gradient followed by prolonged ultracentrifugation.

Procedure for Synaptic Protein Extraction from Primary Cultured Neurons

- Carefully decant culture medium from cells. Wash cells twice with ice-cold PBS.
- Add the appropriate amount of Syn-PER Reagent to the plate or each plate well (see Table 1).

Table 1. Suggested volume of Thermo Scientific Syn-PER Reagent to use for different sizes of standard culture plates.

<u>Plate size/Surface area</u>	<u>Syn-PER Reagent volume</u>
100mm	500-1000 μ L
60mm	250-500 μ L
35mm*	200-400 μ L

*Primary cultured neurons grown for 3 weeks in a 35mm plate containing 10⁶ cells yield ~4.0 μ g of synaptic protein.

- Scrape the plate surface using a cell scraper to lift the cells. Collect the lysate and transfer to a microcentrifuge tube.
- Centrifuge sample at 1200 \times g for 10 minutes at 4°C. Discard the pellet and transfer supernatant to a new tube. If required, save a sample of the supernatant (homogenate) for analysis.
- Centrifuge supernatant at 15,000 \times g for 20 minutes at 4°C.
- Remove the supernatant from the synaptosome pellet. If required, save the supernatant (cytosolic fraction) for analysis.
- Suspend the synaptosome pellet in Syn-PER Reagent; final volume is dependent on the size of the culture dish (e.g., 20-40 μ L per sample for a 35mm dish).
- Maintain the synaptosome suspension on ice until performing neurotransmitter release studies or downstream applications.

Note: The synaptosome suspension can be stored in 5% (v/v) DMSO at -80°C or in liquid nitrogen for extended periods of time; however, a substantial reduction in synaptosome viability occurs after prolonged storage. For best results, perform activity studies (e.g., calcium-dependent neurotransmitter release) with new synaptosomes.

Note: Further synaptosome suspension separation into pre- and post-synaptic protein fractions and synaptic vesicle proteins is done by applying the synaptosome suspension onto a discontinuous sucrose gradient followed by prolonged ultracentrifugation.

Related Thermo Scientific Products

78420	Halt™ Phosphatase Inhibitor Cocktail (100X), 1mL
87785	Halt Protease Inhibitor Cocktail, EDTA-free (100X), 1mL
87786	Halt Protease Inhibitor Cocktail Kit, 1mL
78440	Halt Protease and Phosphatase Inhibitor Cocktail (100X), 1mL
78441	Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X), 1mL
88660	Pierce® Protease Inhibitor Tablets, 30 tablets
88661	Pierce Protease Inhibitor Tablets, EDTA-free, 30 tablets
88662	Pierce Phosphatase Inhibitor Tablets, 20 tablets
88663	Pierce Protease and Phosphatase Inhibitor Tablets, 20 tablets
88664	Pierce Protease and Phosphatase Inhibitor Tablets, EDTA-free, 20 tablets
87791	Pierce Tissue Strainers, 250µm, 50 each
87792	N-PER® Neuronal Protein Extraction Reagent, 100mL
87790	Subcellular Protein Fractionation Kit for Tissues
23225	Pierce BCA Protein Assay Kit
15041	Pierce 96-Well Plates, Corner-notch, 100/pkg
28372	BupH™ Phosphate Buffered Saline Packs, 40 packs
28348	20X Phosphate Buffered Saline, 500mL

General References

Bai, F., *et al.* (2007). Synaptosome proteomics. *Subcell Biochem* **43**:77-98.

Baldwin, M.L., *et al.* (2003). Two modes of exocytosis from synaptosomes are differentially regulated by protein phosphatase types 2A and 2B. *J of Neurochem* **85**:1190-9.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at www.thermoscientific.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

© 2011 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.